Induction of DNA Synthesis in Primary Cultures of Rat Hepatocytes by Serotonin: Possible Involvement of Serotonin S_2 Receptor

SUDHA BALASUBRAMANIAN AND CHERAMADATHIKUDYIL S. PAULOSE

The involvement of serotonin and its receptor subtype in the induction of hepatocyte DNA synthesis was investigated in primary cultures of adult rat hepatocytes. Serotonin caused a dose-dependent increase in DNA synthesis in primary cultures of rat hepatocytes in the presence of epidermal growth factor (EGF) and insulin, as measured by [^{3}H]thymidine incorporation. The serotonin S_2 receptor antagonists, ketanserin (10^{-6} mol/L) and spiperone (10^{-6} mol/L), blocked stimulation of DNA synthesis by serotonin. Displacement studies on [^{3}H]5-hydroxytryptamine (5-HT) binding to crude membranes from control and regenerating liver tissue, using cold ketanserin and spiperone, showed an increased involvement of S_2 receptors of serotonin in the regenerating liver during the DNA-synthetic phase. Serotonin enhanced the phosphorylation of a 40-kd substrate protein of protein kinase C (PKC) in the regenerating liver during the DNA synthetic phase of the hepatocyte cell cycle. This was blocked by ketanserin, indicating that serotonin S_2 receptor activates PKC, an important second messenger in cell growth and division, during rat liver regeneration. Our results show that serotonin can act as a potent hepatocyte comitogen and induce DNA synthesis in primary cultures of rat hepatocytes, which is suggested to be mediated through the serotonin S_2 receptors of hepatocytes. (HEPATOLOGY 1998;27:62-66.)

The adult mammalian hepatocytes are highly differentiated, nonproliferating cells that can be induced to divide after partial hepatectomy. Liver regeneration after partial hepatectomy is a useful model to study the mechanisms involved in the control of cell growth and division.[1] The re-entry of hepatocytes into the cell cycle is characterized by coordinated waves of DNA synthesis, and cell division is terminated by intrinsic control mechanisms when the original cellular mass has been restored.[2] The hepatic regenerative response may be regulated by hormones such as insulin and thyroid hormones,[3-6] and by neurotransmitters such as norepinephrine.[7,8] Adult rat hepatocytes can be induced to enter into DNA synthesis and mitosis in primary culture. Liver regeneration studies with replicating hepatocytes in culture can be used to investigate the trophic factors that control the growth of normal and neoplastic hepatocytes.[9]

The hepatic sympathetic nervous system has been implicated to be important in DNA synthesis during liver regeneration.[10,11] Cruise et al.[12] reported that norepinephrine stimulated DNA synthesis in cultured rat hepatocytes through the α_1-adrenergic receptor. Serotonin has been shown to be mitogenic in many non-neuronal cells, exerting its effect by its different receptor-mediated second messenger pathways. The S_2 receptor subtype of serotonin has been shown to mediate cell growth in fibroblasts.[13] The serotonin S_2 receptor has been cloned in the human liver and has been shown to have a high degree of homology with the S_2 receptors of rat and mouse liver.[14] The S_2 receptors of serotonin are coupled to phospho-inositide turnover and diacylglycerol formation, which activates protein kinase C (PKC), an important second messenger for cell division.[15] Serotonin S_2 receptor–mediated activation of PKC has been shown to result in the phosphorylation of a 40-kd substrate protein of PKC in human platelets.[16] In the present study, the effect of serotonin and the serotonergic S_2 receptor antagonists, ketanserin and spiperone, in induction of DNA synthesis in primary cultures of adult rat hepatocytes was investigated. The changes in the serotonin S_2 receptors and S_2 receptor–mediated membrane protein phosphorylation were also studied in the regenerating rat liver after partial hepatectomy.[1]

MATERIALS AND METHODS

Animals. Adult male Sprague-Dawley rats weighing 200 to 300 g were used for all experiments. They were fed lab chow and water *ad libitum*, and maintained on a 12-hour light/dark cycle.

Materials. [^{3}H]Thymidine (18 Ci/mmol) and [γ-^{32}P]adenosine triphosphate (ATP) (3,000 Ci/mmol) were purchased from BARC (Mumbai, India). Epidermal growth factor (EGF), insulin, serotonin, collagenase type-IV, collagen, fetal calf serum, Williams' medium E, aphidicolin, phosphatidyl serine, dithiothreitol, standard marker proteins (myosin [205 kd], β-galactosidase [116 kd], phosphorylase 97.4 kd], bovine albumin [66 kd], egg albumin [45 kd], and carbonic anhydrase [29 kd]), and other biochemicals were purchased from Sigma Chemical Co. (St. Louis, MO). Ketanserin and spiperone were gifts from Janssen Research Laboratories, Beerse, Belgium. [^{3}H]5-hydroxytryptamine (5-HT) (18.4 Ci/mmol) was from Amersham, Buckinghamshire, UK. All other chemicals were standard commercial products of analytical grade.

Isolation and Culture of Hepatocytes. Hepatocytes were isolated from male Sprague-Dawley rats (range, 200-300 g) by collagenase perfusion, filtration, and low-speed centrifugation as described by

Abbreviations: PKC, protein kinase C; ATP, adenosine triphosphate; EGF, epidermal growth factor; 5-HT, 5-hydroxytryptamine (serotonin); TCA, trichloroacetic acid.

From the Molecular Neurobiology and Cell Biology Unit, Department of Biotechnology, Cochin University of Science and Technology, Cochin, Kerala, India.

Received May 14, 1997; accepted September 16, 1997.

Supported by a research grant from DST, Government of India (to C.S.P.).

Address reprint requests to: Dr. C. S. Paulose, Molecular Neurobiology and Cell Biology Unit, Department of Biotechnology, Cochin University of Science and Technology, Cochin 682022, Kerala, India. Fax: 91-0484-53-2495.

0270-9139/98/2701-001153.00/0

.[17] Livers were perfused with a Ca^{2+}-free HEPES buffer (pH ollowed by the same buffer (pH 7.6) containing 5 mmol/L and 0.05% collagenase. The hepatocyte preparation having a y of >90% as assessed by Trypan Blue exclusion was chosen ture. The cells were plated on 35-mm rat-tail collagen-coated at a density of approximately 10^6 cells in 1 mL per culture ells were allowed to settle and adhere for 3 hours in Williams' m E supplemented with 5% fetal calf serum, gentamycin (50 mg/mL), and insulin (10^{-7} mol/L). After 3 hours (zero f assay), the medium was replaced by serum-free medium ning [^{3}H]thymidine (2.5 mCi/mL) with and without EGF (10). Dose response of hepatocyte DNA synthesis to 5-HT was l by adding varying concentrations of 5-HT (5×10^{-9} mol/L mol/L) to primary cultures of rat hepatocytes in the presence d concentrations of EGF (10 ng/mL) and insulin (10^{-7} . The serotonergic receptor blockers, ketanserin and spipe- 10^{-6} mol/L), were examined for their ability to block the ition of DNA synthesis induced by 5-HT. Cultures were ted for 48 hours in 95% air, 5% CO_2 at 37°C.[12]

Synthesis Assay. After 48 hours, the hepatocytes were har- and DNA was extracted by trichloroacetic acid (TCA) ition according to Takai et al.[18] The cells were washed twice old phosphate-buffered saline, and 1 mL of cold TCA was The hepatocytes were solubilized by incubation at 37°C for nutes in 0.5 mL of 1N NaOH, and then cold 100% TCA was to the solution at a final concentration of 15%, and the tate was washed with 5% TCA. DNA was hydrolyzed by g the precipitate at 90°C for 15 minutes in 0.5 mL of 10% DNA synthesis was measured by [^{3}H]thymidine incorporation NA. Radioactivity was measured in a Wallac liquid scintilla- unter, Turku, Finland. The difference between the radioactivi- the hot TCA-soluble fractions with or without 10 μg/mL colin was expressed as dpm/mg protein. Cell protein was ted according to the method of Lowry et al.[19]

tal Hepatectomy and Killing. Two thirds of the liver constituting dian and left lateral lobes were surgically excised under ether esia according to the method of Higgins and Anderson.[20] operations involved median excision of the bodywall, fol- by all manipulations except removal of the lobes. After 24 of surgery, the animals were killed, and the liver was dissected ored at −70°C.

5-HT Displacement Studies in Liver. Control and regenerating ssue was homogenized in 50 volumes of ice-cold 0.25 mol/L 10 mmol/L Tris-HCl buffer (pH 7.5) containing 2 mmol/L ne glycol-bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid, entrifuged at 4,8000g for 30 minutes. The pellet was resus- d in the same buffer and recentrifuged. The final pellet was pended in the assay buffer and was used as crude membrane ration for displacement studies.[21] Ketanserin and spiperone mol/L to 10^{-3} mol/L) were used to perform competitive g studies with [^{3}H]5-HT. The radioactivity was measured in a liquid scintillation counter.

-Dependent Protein Phosphorylation Assays in Particulate Fractions trol and Regenerating Rat Liver. Control and regenerating liver omogenized in 20 mmol/L TRIS-HCl at pH 7.5 containing 2 L ethylenediaminetetraacetic acid and 10 mmol/L ethylene -bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid to obtain homogenate.[22] The homogenates were centrifuged at 48,000g minutes at 4°C, and particulate fractions were used for the horylation assay. Protein was estimated according to the d of Lowry et al.[19] PKC-dependent phosphorylation assays in articulate fraction of control and regenerating liver were med by a modified procedure of Jaiswal et al.[23] The reaction re (50 mL final volume) consisted of 20 mmol/L TRIS-HCl .5), 5 mmol/L $MgCl_2 \cdot 6H_2O$, 10 mmol/L dithiothreitol, 0.5 /L $CaCl_2 \cdot 2H_2O$, and 10 mg phosphatidyl serine, and 50 mg n of particulate preparation. The compounds, 10^{-5} mol/L ntration of 5-HT, and ketanserin were added appropriately to phosphorylation. After incubation with the various drugs at 0°C for 15 minutes, the reaction was started by adding 10 mmol/L ATP containing 1 mCi of [γ-^{32}P]ATP, and further incubated at 30°C for 2 minutes. The reaction was terminated by adding 15 mL of 4× stop buffer (250 mmol/L TRIS-HCl [pH 6.8], 8% sodium dodecyl sulfate, 40% glycerol, and 20% β-mercaptoethanol). The samples were transferred to a boiling water bath for 2 minutes. Proteins were resolved by sodium dodecyl sulfate–polyacrylamide gel electrophoresis on a 10% polyacrylamide gel by Laemmli's method.[24] Standard marker proteins were simultaneously electrophoresed. The gels were stained with Coomassie blue R-250, dried on a gel drier (Hoeffer, San Francisco, CA), and autoradiographed on Kodak X-ray films with intensifying screens at −70°C for 24 hours.

RESULTS

EGF (10 ng/mL), a known hepatocyte mitogen, resulted in a significant increase ($P < .05$) in DNA synthesis of cultured hepatocytes in the presence of insulin. When serotonin alone was added to hepatocyte cultures, it did not bring about a significant increase in the DNA synthesis in the presence of insulin. However, serotonin significantly enhanced ($P < .05$) DNA synthesis of hepatocytes in the presence of EGF and insulin (Table 1). Dose-response studies with serotonin showed that the effective concentration of serotonin was 10^{-6} mol/L and the maximal effect was reached at 5×10^{-5} mol/L (Fig. 1). Serotonergic inhibitors were examined for their ability to block the stimulation of DNA synthesis induced by 5×10^{-5} mol/L 5-HT (Table 1). Ketanserin, at 10^{-6} mol/L, caused a significant reduction in 5-HT–induced DNA synthesis ($P < .05$). The addition of 10^{-6} mol/L of spiperone also led to a significant decrease in DNA synthesis induced by 5-HT ($P < .05$). Thus, ketanserin and spiperone were able to block the co-mitogenic effect of serotonin.

Displacement studies were performed as *in vitro* assays on crude membrane preparations of control and regenerating liver. Ketanserin caused a marked displacement of [^{3}H]serotonin from its receptors in the regenerating rat liver at 24 hours after partial hepatectomy compared with control liver, in all concentrations tested (Fig. 2). Spiperone displaced [^{3}H]serotonin in the low-affinity range of concentrations during the DNA synthetic phase (Fig. 3). Both antagonists caused a shift of the displacement curve to the high-affinity concentration range.

The membrane proteins phosphorylated by 5-HT in a PKC-dependent manner were studied in crude membrane preparations of the control and 24-hour regenerating rat liver (Fig. 4). Endogenous PKC-dependent phosphorylation of a

TABLE 1. Effect of Serotonin and Serotonergic Receptor Antagonists on DNA Synthesis in Primary Cultures of Rat Hepatocytes

Experiment	DNA Synthesis (dpm/mg protein × 10^{-3})
Medium only	23.53 ± 0.68
50 μmol/L 5-HT	27.46 ± 0.24
EGF (10 ng/mL)	451.89 ± 7.81*
EGF + 50 μmol/L 5-HT	852.41 ± 9.35*
EGF + 50 μmol/L 5-HT + 1 μmol/L ketanserin	334.18 ± 14.57*
EGF + 50 μmol/L 5-HT + 1 μmol/L spiperone	375.69 ± 9.86*

NOTE. Values are mean ± SEM of four to six separate determinations (Duncan's multiple range test). Hepatocyte culture and assay of DNA synthesis were performed as described in Materials and Methods. Insulin (10^{-7} mol/L) was present in all cultures.

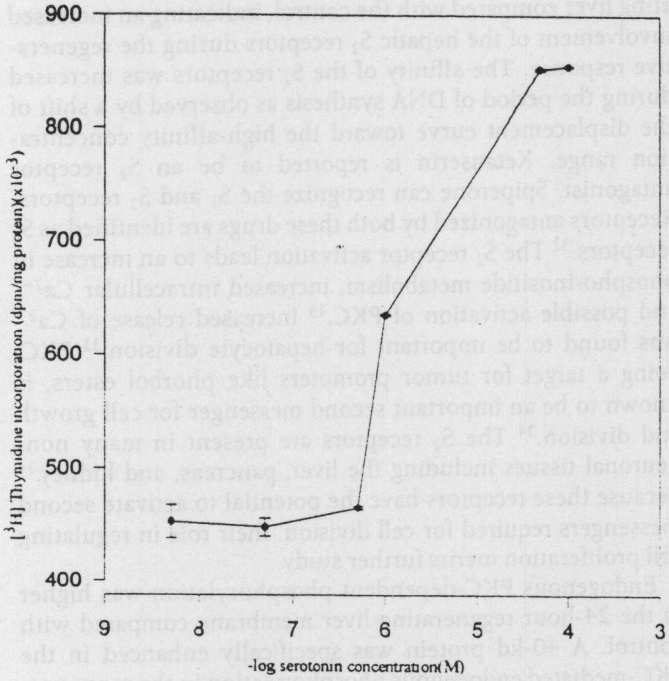


FIG. 1. Dose-dependent response of DNA synthesis in primary cultures of rat hepatocytes to serotonin. Different concentrations of serotonin (5×10^{-8} mol/L-10^{-4} mol/L) were added to cultured hepatocytes, and [³H]thymidine incorporation was determined in the presence of EGF (10 ng/mL) and insulin (10^{-7} mol/L). Values are mean ± SEM of four to six separate determinations. Where the ERROR BARS are not visible, the SEM is <2%.

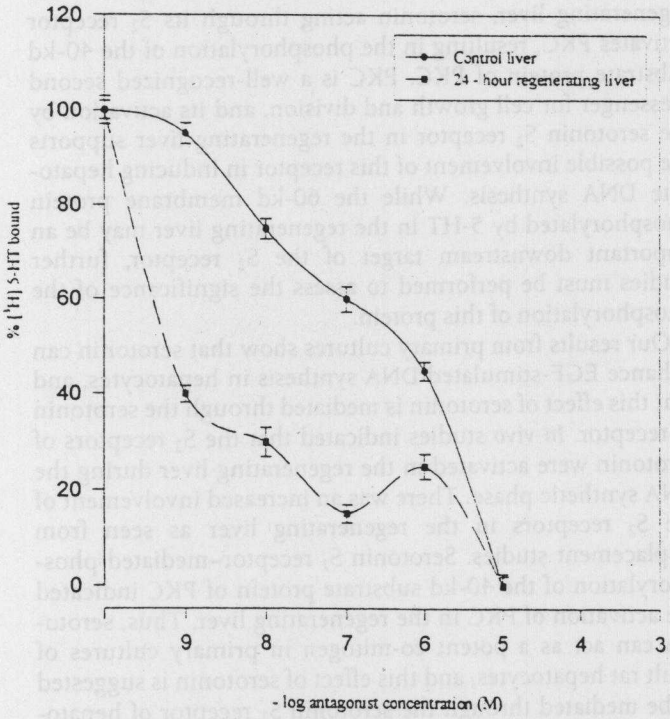


FIG. 2. Displacement analysis of [³H]5-HT by ketanserin in crude liver membrane preparations of control and hepatectomized rats assayed *in vitro*. Competitive binding studies were performed with 3 nmol/L of [³H]5-HT and 10^{-9} mol/L to 10^{-5} mol/L cold ketanserin. Values are mean ± SEM of four to six separate determinations and are plotted at different concentrations.

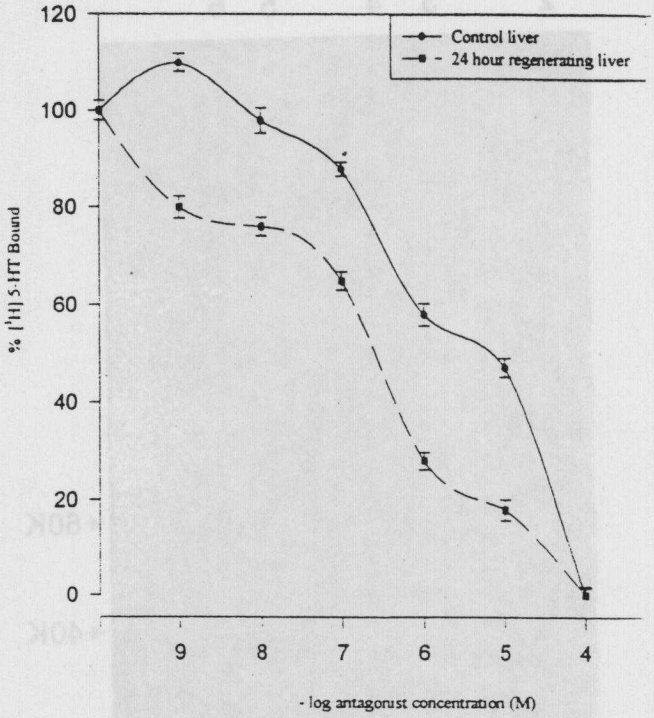
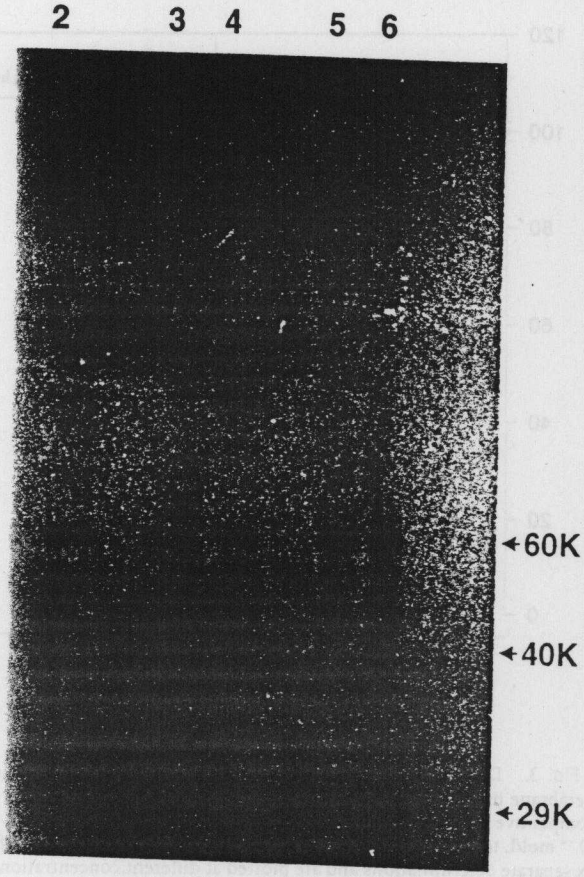


FIG. 3. Displacement analysis of [³H]5-HT by spiperone in crude l membrane preparations of control and hepatectomized rats assayed *in v* Competitive binding studies were performed with 5 nmol/L of [³H]5-HT 10^{-9} mol/L to 10^{-5} mol/L cold spiperone. Values are mean ± SEM of fou six separate determinations and are plotted at different concentrations.

29-kd and a 40-kd membrane protein was higher in t 24-hour regenerating liver membrane compared with contr Additional phosphorylation of a 50-kd protein and a 60- protein was also seen in the regenerating liver membrane hours after partial hepatectomy. When 5-HT was adde phosphorylation of the 40-kd membrane protein in t regenerating liver was enhanced compared with the cont liver. When ketanserin was added along with 5-HT, there w a conspicuous decrease in the phosphorylation of the 40- protein in the control and regenerating liver membrane. T phosphorylation of the 60-kd membrane protein, which w markedly enhanced when 5-HT was added, was blocked the control and regenerating liver membrane fractions in t presence of ketanserin.

DISCUSSION

The results of this study showed that serotonin induc DNA synthesis of mature rat hepatocytes in primary cultu and this effect of serotonin is suggested to be mediated by t S_2 receptor of serotonin. Quiescent hepatocytes in cultu were stimulated to enter the DNA synthetic phase by E(Though serotonin was nonmitogenic *per se*, it induced D synthesis in the presence of EGF. Polypeptide growth fact such as EGF have been defined as complete mitogens hepatocytes.[25,26] Insulin, EGF, and glucagon have been sho to elicit DNA synthesis in cultures of hepatocytes. Insulin required for the full magnitude of the response duri EGF-mediated DNA synthesis.[27] The plasticity of grow responses seen in hepatocytes may be controlled by comit genic substances such as neurotransmitters. Studies on t role of neurotransmitters as modulators of hepatocyte di



Autoradiograph of 5-HT–induced membrane protein phosphory-
ketanserin effect to show the S_2 receptor involvement in the
d regenerating rat liver. Crude liver membrane protein fractions
rol and regenerating liver were used in four to six separate
ts. Fifty micrograms of protein was incubated with 10^{-5} mol/L
ions of the drugs and [γ-^{32}P]ATP. Protein was quantified by
thod. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis
med as described in Materials and Methods. *Lane 1*, endogenous
lation in the control liver; *lane 2*, endogenous phosphorylation in
rating liver; *lane 3*, 5-HT with ketanserin in the control liver; *Lane*
th ketanserin in the regenerating liver; *lane 5*, with 5-HT in control
6, with 5-HT in regenerating liver.

e focused on norepinephrine effects. Norepinephrine
n shown to antagonize the inhibitory effects of
ming growth factor β (TGF-β) on DNA synthesis of
rat hepatocytes.[28] Norepinephrine enhanced the
city of EGF by causing a down-regulation of EGF
s.[8]

transmitter receptors are usually restricted to neuro-
. However, neurotransmitters have been shown to
e or inhibit proliferation of non-neuronal cells by
g receptors coupled to different second messenger
s.[29] Serotonin has been found to promote cell prolif-
n various cell types. In aortic smooth muscle cells,
-induced mitogenesis was comparable with that of
latelet–derived growth factor.[30] The serotonin 5-HT_{1C}
has been reported to function as a proto-oncogene in
fibroblasts, in which its expression triggers malig-
sformation.[31]

, at 24 hours of liver regeneration, when the DNA
s is markedly elevated,[2] ketanserin and spiperone
significant displacement of [^{3}H]5-HT in the regener-
ating liver compared with the control, indicating an increased involvement of the hepatic S_2 receptors during the regenerative response. The affinity of the S_2 receptors was increased during the period of DNA synthesis as observed by a shift of the displacement curve toward the high-affinity concentration range. Ketanserin is reported to be an S_2 receptor antagonist. Spiperone can recognize the S_1 and S_2 receptors. Receptors antagonized by both these drugs are identified as S_2 receptors.[32] The S_2 receptor activation leads to an increase in phospho-inositide metabolism, increased intracellular Ca^{2+}, and possible activation of PKC.[15] Increased release of Ca^{2+} was found to be important for hepatocyte division.[33] PKC, being a target for tumor promoters like phorbol esters, is known to be an important second messenger for cell growth and division.[34] The S_2 receptors are present in many non-neuronal tissues including the liver, pancreas, and kidney.[14] Because these receptors have the potential to activate second messengers required for cell division, their role in regulating cell proliferation merits further study.

Endogenous PKC-dependent phosphorylation was higher in the 24-hour regenerating liver membrane compared with control. A 40-kd protein was specifically enhanced in the PKC-mediated endogenous phosphorylation in the regenerating liver membrane. Previous studies have shown that, in human platelets, serotonin phosphorylates a 40-kd protein that was reported to be the substrate protein of PKC. This was shown to be mediated by the S_2 receptor of serotonin.[16] In our study, we also observed that 5-HT enhanced the PKC-dependent phosphorylation of a 40-kd membrane protein in the regenerating liver. Ketanserin, the S_2 receptor blocker, brought about a decrease in the 5-HT–induced phosphorylation of this protein. This serves as evidence that, in the regenerating liver, serotonin acting through its S_2 receptor activates PKC, resulting in the phosphorylation of the 40-kd substrate protein of PKC. PKC is a well-recognized second messenger for cell growth and division, and its activation by the serotonin S_2 receptor in the regenerating liver supports the possible involvement of this receptor in inducing hepatocyte DNA synthesis. While the 60-kd membrane protein phosphorylated by 5-HT in the regenerating liver may be an important downstream target of the S_2 receptor, further studies must be performed to assess the significance of the phosphorylation of this protein.

Our results from primary cultures show that serotonin can enhance EGF-stimulated DNA synthesis in hepatocytes, and that this effect of serotonin is mediated through the serotonin S_2 receptor. *In vivo* studies indicated that the S_2 receptors of serotonin were activated in the regenerating liver during the DNA synthetic phase. There was an increased involvement of the S_2 receptors in the regenerating liver as seen from displacement studies. Serotonin S_2 receptor–mediated phosphorylation of the 40-kd substrate protein of PKC indicated the activation of PKC in the regenerating liver. Thus, serotonin can act as a potent co-mitogen in primary cultures of adult rat hepatocytes, and this effect of serotonin is suggested to be mediated through the serotonin S_2 receptor of hepatocytes.

Acknowledgment: Sudha Balasubramanian thanks the Council of Scientific and Industrial Research, New Delhi, India, for the Senior Research Fellowship.

REFERENCES

1. Taub R. Transcriptional control of liver regeneration. FASEB J 1996;10: 413-427.
2. Grisham JW. A morphologic study of deoxyribonucleic acid synthesis and cell proliferation in the regenerating liver: autoradiography with thymidine-H3. Cancer Res 1962;22:842-849.
3. Mola PW, Sudha B, Paulose CS. Effect of insulin on DNA synthesis and kinetic parameters of thymidine kinase during liver regeneration. Biochem Mol Biol Int 1996;40:1067-1075.
4. Maliekal TT, Sudha B, Paulose CS. Kinetic parameters of thymidine kinase and DNA synthesis during rat liver regeneration: role of thyroid hormones. Life Sci 1997;60:1867-1874.
5. Bucher NLR, Swaffield MN. Regulation of hepatic regeneration in rats by synergistic action of insulin and glucagon. Proc Natl Acad Sci U S A 1975;72:1157-1160.
6. Bucher NLR, Patel U, Cohen S. Hormonal factors and liver growth. Adv Enz Regul 1978;16:205-213.
7. Cruise JL, Michalopoulos G. Norepinephrine and epidermal growth factor: dynamics of their interaction in the stimulation of hepatocyte DNA synthesis. J Cell Physiol 1985;125:45-50.
8. Cruise JL, Cotecchia S, Michalopoulos G. Norepinephrine decreases EGF binding in primary rat hepatocyte cultures. J Cell Physiol 1986;127: 39-44.
9. Michalopoulos G, Cianciulli HD, Novotny AR, Kligerman AD, Strom SC, Jirtle RL. Liver regeneration studies with rat hepatocytes in primary culture. Cancer Res 1982;42:4673-4682.
10. Morley CGD, Royse VL. Adrenergic agents as possible regulators of liver regeneration. Int J Biochem 1981;13:969-973.
11. Kiba T, Tanaka K, Inoue S. Lateral hypothalamic lesions facilitate hepatic regeneration after partial hepatectomy in rats. Pflug Arch Eur J Physiol 1995;430:666-671.
12. Cruise JL, Houck KA, Michalopoulos GK. Induction of DNA synthesis in cultured rat hepatocytes through stimulation of α_1 adrenoreceptor by norepinephrine. Science 1985;227:749-751.
13. Van Obberghen-Schilling E, Vouret-Craviari V, Haslam, RJ, Chambard JC, Pouyssegur JM. Cloning, functional expression and role in cell growth regulation of a hamster 5-HT_2 receptor subtype. Mol Endocrinol 1991;5:881-889.
14. Bonahus DW, Bach C, De Souza A, Salazar FH, Matsuoka BD, Zuppan P, Chan HW, et al. The pharmacology and distribution of human 5-hydroxytryptamine 2B (5-HT_{2B}) receptor gene products: comparison with 5-HT_{2A} and5-HT_{2C} receptors. Br J Pharmacol 1995;115:622-628.
15. DeCourcelles CD, Leysen EJ, De Clerck F, Van Belle H, Janssen AP. Evidence that phospholipid turnover is the signal transducing system coupled to serotonin-S_2 receptor sites. J Biol Chem 1985;260:7603-7608.
16. DeCourcelles CD, Roevens P, Van Belle H. Stimulation by serotonin of 40KDa and 20 KDa protein phosphorylation in human platelets. FEBS Lett 1984;171:289-292
17. Seglen PO. Preparation of isolated rat liver cells. Methods Cell Biol 1976;13:29-83.
18. Takai S, Nakamura T, Komi N, Ichihara A. Mechanism of stimulation of DNA synthesis induced by epinephrine in primary cultures of adult rat hepatocytes. J Biochem 1988;103:848-852.
19. Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ. Protein measurement with Folin-Phenol reagent. J Biol Chem 1951;193:265-275.
20. Higgins GM, Anderson RM. Experimental pathology of the liver. I Restoration of the liver of the white rat following partial surgical removal. Arch Pathol 1931;12:186-202.
21. Paulose CS, Dakshinamurti K. Effect of pyridoxine deficiency in young rats on high-affinity serotonin and dopamine receptors. J Neurosci Res 1985;14:263-270.
22. Kikkawa U, Takai Y, Minakuchi R, Inohara S, Nishizuka Y. Calcium activated, phospholipid-dependent protein kinase from rat brain. J Biol Chem 1982;257:13341-13348.
23. Jaiswal AS, Misra UK, Bansal SK. Differential activity of protein kinase C in alveolar and peritoneal macrophages. Int J Biochem Biophys 1996;33 116-121.
24. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 1970;227:680-685.
25. Michalopoulos GK. Liver regeneration: molecular mechanisms of growth control. FASEB J 1990;4:176-187.
26. Vintemyr KO, Doskeland OS. Cell cycle parameters of adult rat hepatocytes in a defined medium: a note on the timing of nucleolar DNA replication. J Cell Physiol 1987;132:12-21.
27. McGowan JA, Strain AJ, Bucher NLR. DNA synthesis in primary cultures of adult rat hepatocytes in a defined medium: effects of epidermal growth factor, insulin, glucagon and cyclic-AMP. J Cell Physiol 1981;108 353-363.
28. Houck KA, Cruise JL, Michalopoulos G. Norepinephrine modulates the growth inhibitory effects of transforming growth factor-beta in primary rat hepatocyte cultures. J Cell Physiol 1985;135:551-555.
29. Lauder JM. Neurotransmitters as growth regulatory signals: role of receptors and second messengers. Trends Neurosci 1993;16:233-240.
30. Nemeck GM, Coughlin SR, Handley AD, Moskowitz AM. Stimulation of aortic smooth muscle cell mitogenesis by serotonin. Proc Natl Acad Sci U S A 1986;83:674-678.
31. Julius D, Livelli JT, Jessell MT, Axel R. Ectopic expression of the serotonin 1c receptor and the triggering of malignant transformation Science 1989;244:1057-1062.
32. Hoyer D, Clarke DE, Fozard RJ, Hartig RP, Martin RG, Mylecharane JE Saxena RP, et al. International Union of Pharmacology classification of receptors for 5-hydroxytryptamine (serotonin). Pharmacol Rev 1994;46 157-203.
33. Rixon RH, Isaacs RJ, Whitfield FJ. Control of DNA polymerase activity in the regenerating liver by calcium and 1a,25 $(OH)_2$ D_3. J Cell Physiol 1989;139:354-359.
34. Weinstein IB. Protein kinase, phospholipid and control of growth Nature 1983;302:750.